SYNOPSIS

Submitted to Trans-disciplinary University (TDU)

ON

"STUDY ON IMPACT OF ETHNOVETERINARY HERBAL PREPARATIONS IN BOVINE DISEASES TO MINIMISE USE OF ANTIMICROBIAL DRUGS"

Synopsis submitted

in Partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

2023

Co-supervisor	**Co-supervisor**	**Supervisor**
Dr. MNB Nair PhD	Dr. SK Rana PhD	Dr. N Punniamurthy PhD
Centre Head Prof. Emeritus TDU	World Bank Consultant Ex. Mission Director NDLM, NDDB	NDDB Consultant (AH group) Prof. Emeritus, TDU

SUBMITTED BY

Dr. Pankaj Dutta

Regn. No: 20318020228

A study on the impact of ethnoveterinary herbal preparations (EVHP) in bovine diseases was carried out for the doctoral degree at TDU, to ascertain the possibility of reducing the use of antimicrobial drugs for the common ailments of bovine and to combat the antimicrobial resistance issues (AMR), with the following objectives:

A. To conduct a survey on awareness of farmers on the use of Ethno-veterinary Herbal Preparations (EVHP) with special reference to common diseases of dairy animals.

B. Studies on causative bacterial agents of bovine mastitis, identify and assessing antibiotic resistance pattern in these agents.

C. To study the efficacy of EVHP in the management of bovine mastitis.

D. Case studies on the recovery of clinical mastitis by using EVHP under field condition.

E. To study the effect of using EVHP in bovine diseases on antibiotic residues in milk in the study villages.

Additional studies carried out:

F. Field studies on the recovery from LSD, Repeat Breeding, Pyrexia and Diarrhoea by using EVHP under field conditions
G. The effect of using EVHP in bovine disease on somatic cell counts in bulk milk samples in the study villages.
H. *In-vivo* dermal safety study of mastitis EVHP preparation in lab animal

Works done

- Studies on the farmers' awareness on EVHP was undertaken among the dairy farmers from two milk unions and two producer companies. Data obtained was analysed and presented.
- Detection of antibiotic residue (covering 10 villages) and SCC (covering 15 villages) in bulk milk was carried out in Sabarkantha Milk Union.
- Bacterial isolation, identification, antibiogram, molecular characterization, physical properties of milk constituents were carried out at NDDB Laboratory.

- Field studies on the efficacy of EVHP for the management of bovine mastitis, diarrhoea, pyrexia, repeat breeding and LSD was conducted at various milk unions.
- Dermal toxicity in experimental was carried out for the EVHP preparation for mastitis in an NABL accredited Laboratory.
- Eight articles/abstracts published. Two abstracts were presented. Participated in 11 scientific events.

Results and Discussion:

Survey on awareness on bovine mastitis and use of ethnoveterinary herbal preparation (EVHP):

The awareness survey was conducted in four milk union/producer companies.

In Initial survey in Sabarkantha District Co-operative Milk Producers' Union Limited (Sabar Dairy), 50 respondents were surveyed where 92.0 % were aware regarding use of EVHP in cattle and buffaloes. In addition, 77.6 % respondents considered EVHP as cost effective and easily available option. Awareness regarding availability of EVHP for bovine ailments like mastitis, diarrhoea, fever, repeat breeding and FMD were found in between 22.0-78.0 %.

76.0 % respondents were found to be aware regarding possibility of presence of residues in milk after use of antibiotic in animals. Level of aware on EVHP among farmers are shown in Figure-1.

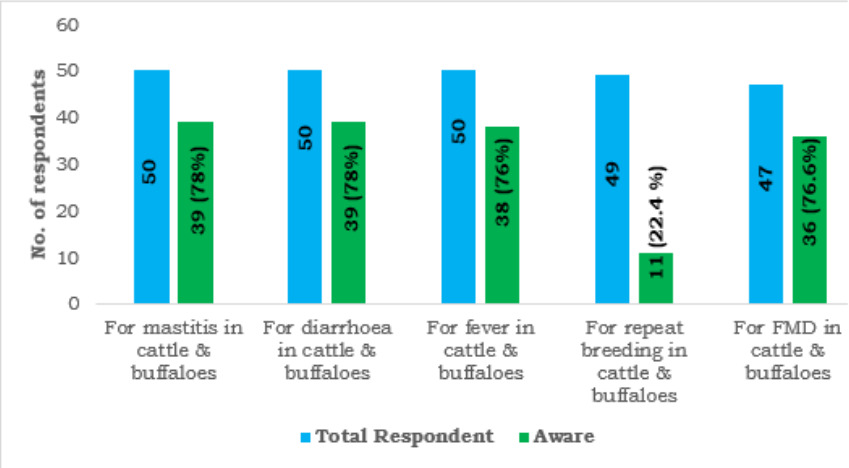


Figure 1. Level of awareness regarding availability of EVHP for various bovine ailments in Sabar Dairy

The subsequent survey was conducted in three organisations i.e. Banni Milk Producer Company Limited, Punjab i.e. Baani MPC, Saahaj Milk Producer Company Limited, Uttar Pradesh i.e. Saahaj MPC and West Assam Milk Producers' Co-operative Union Limited, Assam (WAMUL). This study was comprised of pre and post intervention survey having gap of a year in between. Awareness camp on mastitis and EVHP was conducted by the team of veterinarians under Disease Control through Alternate Method (DCAM) programme during the gap.

A total of 318 dairy farmers during pre-intervention and 215 dairy farmers during post-intervention were included in the study.

The level of overall awareness in respondents regarding mastitis (sub-clinical) in cattle and buffaloes in the milk shed areas increased from 9.1 % to 85.6 %. The increased awareness regarding use of EVHP on mastitis and about presence of antibiotic residue in milk in cattle and buffaloes in the milk shed areas were increased from 12.3 % to 45.2 % and 19.2 % to 74.6 % respectively.

It was observed from the survey conducted in various MU/MPCs that the awareness campaign had a great impact in increasing awareness among the dairy farmers on the significance of mastitis, use of cost effective, efficacious EVHP and presence of antibiotic residues in milk due to indiscriminate use of antibiotics.

In India, around seventy million farmers depend on dairying (Chauhan *et al.*, 2018). Several researchers have also recorded that in small scale dairy farming, non-judicious use of antibiotics in milch animals and lack in focus on milk quality is quite common (Chauhan *et al*., 2018, Mutua *et al*., 2020, Sharma *et al*., 2020). Chelkeba *et al.* (2016) highlighted the significance of widespread extension in rural areas through training, publications, experience sharing in enhancing their awareness on sub-clinical mastitis (SCM). Bhatt *et al.* (2014) investigated on ethnoveterinary practices at Junagarh, Gujarat among farmers which revealed their successful use of different medicinal plants for 13 different animal ailments like gastrointestinal issues, skin-related disorders, respiratory problems, wound healing etc.

Isolation, identification and antibiogram of the bacterial agents from mastitic milk:

A total of 215 milk samples, collected from suspected cases of bovine mastitis were subjected for bacteriological isolation from which 202 bacterial isolates were recovered. Out of 202 isolates, 164 nos. were identified using the BD phoenix M50 instrument

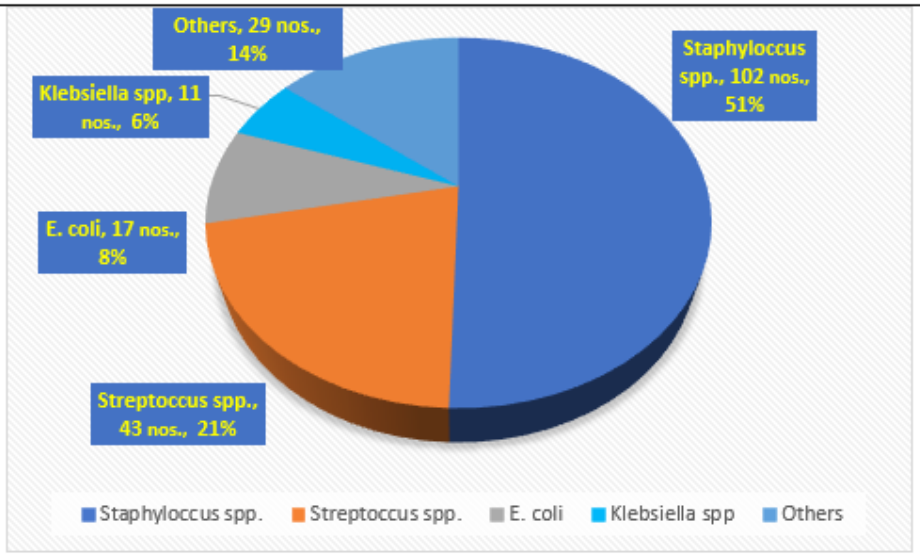
(following manufacturer's manual) and remaining 38 were identified through conventional bacterial identification techniques.

Out of the isolates identified through BD phoenix M50 instrument, 97 isolates were processed for characterization through the Gram-positive combo panel (PMIC/ID-70), 40 isolates through SMIC /ID 9 combo panel for streptococcus and the remaining 27 isolates were screened using the Gram-negative panel (NMIC/ID-55).

Thirty-eight isolates were identified through conventional identification technique due to unavailability of BD system during that period. The identification was carried out up-to genus level except *E. coli.*

Species-wise distribution of isolates revealed, *Staphyloccus* spp. was maximum (51 %) followed by *Streptoccus* spp. (21 %), *E. coli* (17.8 %), *Klebsiella* spp. (6 %) and others (14 %) (Figure-2). Thorberg *et al*., 2009, Hegde *et al.* 2013 and Preethirani *et al.* 2015 had also recorded similar pattern in bacterial isolation from mastitis milk involving *Staphylococcus spp., Streptococcus spp. and E. coli.* In India, *Staphylococcus* spp. have been reported to be the leading etiological agents of mastitis in cattle and buffaloes. (NAAS 2013).

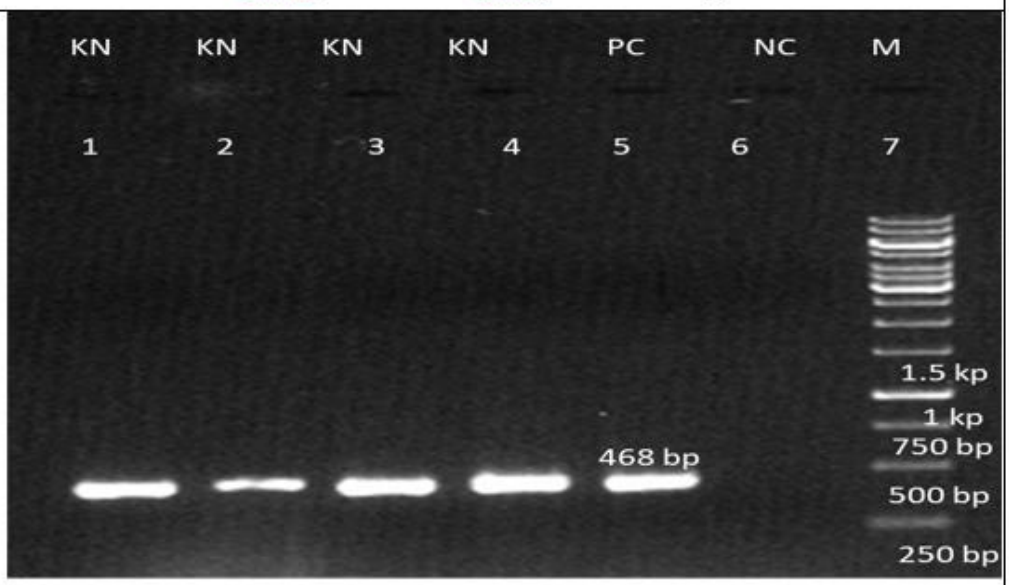
Figure 2: Distribution of overall bacterial agents isolated from bovine mastitis cases (n=202)



Confirmation of bacterial isolates by Polymerase Chain Reaction (PCR)

A representative number of 60 bacterial isolates were further subjected to species specific PCR available for 6 different bacterial species i.e. *E. coli, Kleb. pneumoniae, Staph. aureus, Str. agalactiae, Str. dysgalactiae* and *Str. uberis.* All of the isolates had a 100% match in species identification between the BD system and the PCR data. Species-Specific PCR agarose gel electrophoresis image of confirmed isolates of *Kleb. pneumoniae is* depicted in Figure 3.

Figure 3: Agarose gel electrophoresis image depicting the presence of gyrA gene in *Kleb. pneumoniae* **(kleb primer: 468bp)**



Lane 1, 2, 3 and 4 : *Kleb. pneumoniae (KN), Lane 5:* Positive control (PC), Lane 6: Nigative Control (NC), M: 1 kb Marker,

Antibiogram of bacterial isolates:

The BD Phoenix M50 equipment was used to perform an antibiogram on 123 isolates. Conventional disc diffusion test was performed on 17 isolates where BD Phoenix kit was not available.

Strains of *Staph. aureus* isolated from mastitic milk showed highest degree of resistance to Cefazolin, Norfloxacin (18.4 % each) followed by Oxacillin (17.9 %), Erythromycin, Gentamicin (12.8 % each), Trimethoprim- Sulfamethoxazole (10.3 %), Clindamycin, Quinupristin-dalfopristin, Tetracycline (5.1 % each), Linezolid, Rifampicin, Teicoplanin and Vancomycin (2.6 % each). None of these isolates were

resistant to Daptomycin and Nitrofurantoin. Neelam *et al.* (2022) has recorded maximum resistance of *Staph. aureus* isolates from milk sample to oxytetracycline, penicillin, oxacillin, gentamicin, enrofloxacin, lincomycin and minimum resistance to methicillin and cefuroxime antimicrobials.

The strains of *E coli* isolated from mastitic milk showed highest degree of resistance to Ampicillin (50 %) followed by Tetracycline, Trimethoprim-Sulfamethoxazole (37.5 % each); Amoxicillin-Clavulanate, Cefepime, Aztreonam, Cefoxitin, Ceftazidime, Ceftazidime, Piperacillin (25 % each) and Cefotaxime, Ciprofloxacin, Gentamicin, Levofloxacin (12.5 % each). No resistance was observed for Amikacin, Chloramphenicol, Imipenem and Piperacillin-Tazobactam. Sadashiv and Kaliwal (2015) found resistant *E. coli* strains for Cefpodoxime (62.88%), Ampicillin (49.48%), Cefaclor and Ceftriaxone (42.26%), Cefixime (38.14%), Amoxyclav and Gentamicin (31.95%), Amikacin (23.71%), Chloramphenicol (9.27%), Tetracycline (7.21%), Ciprofloxacin (4.12%). Although, in present study, no resistance was recorded for Amikacin and Chloramphenicol.

The strains of *Kleb. pneumoniae* isolated from mastitic milk showed highest degree of resistance to Ampicillin (100 %) followed by Aztreonam, Cefepime, Cefoxitin, Ceftazidime, Trimethoprim- Sulfamethoxazole (11.11 % each). No resistance was observed for Amikacin, Amoxicillin-Clavulanate, Chloramphenicol, Cefotaxime, Ciprofloxacin, Gentamicin, Levofloxacin Imipenem, Piperacillin, Piperacillin-Tazobactam and Tetracycline. Yadav *et al.* (2021) observed resistance of *Kleb. pneumoniae isolates* against penicillin group of antibiotics, streptomycin and aminoglycosides such as amikacin, neomycin and kanamycin. They recorded susceptibility of the isolates to chloramphenicol, oxytetracycline, gentamicin and levofloxacin which are in agreement with the findings in the present study.

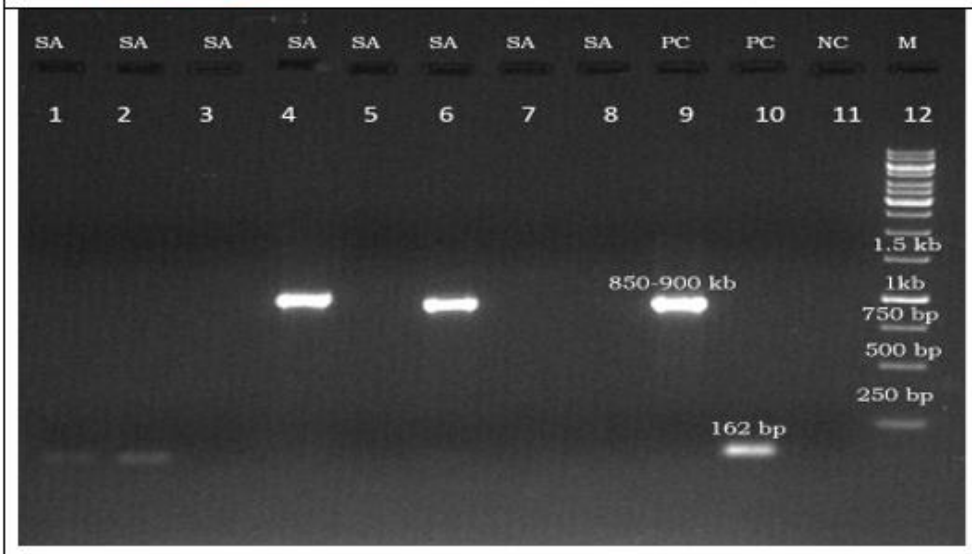
Screening of Antimicrobial Resistant (AMR) genes by PCR:

All the 60 representative bacterial isolates belonging to 6 species were genotyped for the presence of various ARGs. In the present study, screening was carried out for bla_Z, bla_tem_all, bla_SHV, bla_CTX_M, mecA, mecC, TeT-K, TeT-M, TeT-O and TeT-A ARGs, however presence of only bla_Z, bla_SHV, bla_CTX_M, mecA, TeT-K and TeT-M could be detected.

Presence of ARGs i.e. mecA (in 4 of 32 isolates of *Staph. aureus)*, bla-Z (in 4 of 32 isolates of *Staph. aureus)*, bla-SHV (in 6 of 8 isolates of *Kleb. pneumoniae)*, bla CTX-M (in 2 of 6 isolates of *Escherichia coli)*, tet-K (in 5 of 32 isolates of *Staph. aureus* and tet-M (*Str. agalactiae*, 1 of 6 isolates; *Str. uberis, 5 of 5 isolates)* were obtained.

*A*garose gel electrophoresis image depicting the presence of ARGs mecA and bla-Z in confirmed isolates of *Staph. aureus in Figure 4.*

Figure 4: *Agarose* gel electrophoresis image depicting the presence and absence of ARGs mecA and bla-Z in confirmed isolates of *Staph. aureus (162 and 850-900 bp)*



Lane 1-8 : *Staph. aureus (SA),* Lane 9, 10: Positive control (PC), Lane 11: Nigative Control (NC), Lane 12: 1 kb Marker (M)

Isolation and identification of Bacterial agents from milk samples collected before and after EVHP:

Thirty-nine animals were treated with EVHP for mastitis with a recovery rate of 79.5 %. Milk samples from 41 quarters of these animals were collected before EVHP application for bacteriological examination.

The bacterial isolates from pre-EVHP milk samples of 8 animals (out of 39 animals) were screened for presence of AMR genes i.e. mecA, mecC, bla_Z, bla_TEM-all, bla-SHV, bla-CTX-M, Tet-A, Tet-K, Tet-O and Tet-M. Eleven bacterial isolates were recovered from quarter milk samples of these eight animals. Presence of Tet-M gene was recorded in the pre-EVHP milk samples of 4 of these animals.

Therefore, it is obvious that recovery of mastitis with EVHP probably not influenced by presence of resistance genes in isolates recovered from mastitic milk. However, a large-scale study would be required for better observation as well as for reaching to a firm conclusion.

Findings in the present study are agreement to Hegde *et al.* (2021) where they analysed that mastitis cases involving *Staphylococcus, Streptococcus, Pseudomonas, Klebsiella and Enterobacteriaceae family could be* successfully managed by the use of this herbal combination.

Case studies on EVHP applications in different MU/MPCs:

Fifty Holstein Friesian (HF) crossbred cows at Sabarkantha Milk Union affected with SCM were treated with Mastitis EVHP. Around 88% of these SCM positive cows turned CMT negative after completion of EVHP application.

In addition to these 50 Sub-clinical Mastitis (SCM) cases; 3703 clinical mastitis cases, 2240 cases of diarrhoea and 1627 cases of non-specific pyrexia were treated with EVHP with 88 %, 92%, 97.4% and 97.79% recovery rate respectively.

Fourteen Lumpy skin Disease (LSD) and 108 repeat breeder cases (77 % success) were also successfully managed with EVHP.

In the present study, a high rate of clinical recovery was recorded for different ailments/ symptoms with the use of EVHP which clearly indicates that EVHP may play a major role in the management of bovine ailments in India. Similar observations were also recorded by Nair *et al.* (2017) indicating that a combination of Aloe vera 250 g, Curcuma longa 50 g and calcium hydroxide 15 g (NDDB Dairy Knowledge Portal, EVM booklet) is effective in managing bovine mastitis. Satheshkumar *et al.* (2021) recorded an overall 57.1% conception rate among the post-partum anoestus cows treated with EVM (NDDB Dairy Knowledge Portal, EVM booklet). Bhatt *et al*., 2014; Nair *et al.*, 2017; Aruna *et al*. 2019 have also reported that ethno-veterinary medicines were effective against bovine mastitis cases. Verma (2014) documented the use of ethno-veterinary medicinal plants by rural farmers and traditional herbal healers of villages at Bundelkhand region for different animal ailments including diarrhoea and fever.

EVHP intervention in SCM, clinical mastitis, diarrhoea, pyrexia, LSD, and in repeat breeders showed very encouraging results without the use of synthetic medications. With a cost-efficient and effective alternative animal disease control strategy, EVHP has great potential in developing country like India where the resources available to stakeholders are constrained. Additionally, this reduces the amount of drug residues in milk and milk products, which stall the emergence of AMR.

Empirical data recorded in NDDB portal by veterinarians from different states for various bovine ailments treated by EVHP:

EVHP therapy being carried out in the field across the country in the mastitis control project areas have been captured online in which a total of 7,59,792 empirical cases of various ailments with overall 80 % clinically cure rates have been recorded. Disease wise break have been depicted in Figure-5.

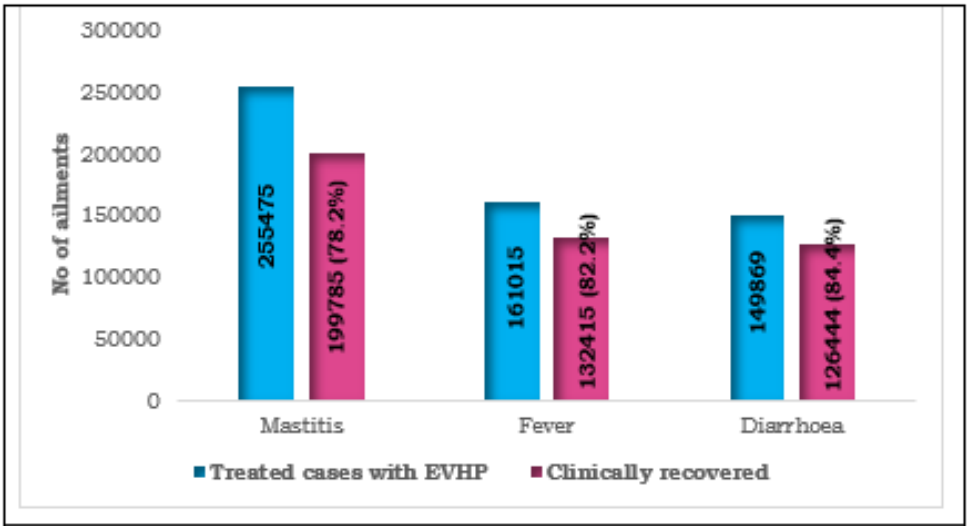


Figure 5: No of bovine ailments managed by EVHP with >100,000 empirical data

Effect of using EVHP in bovine diseases on antibiotic residues in bulk milk in the study villages:

Screening for presence of antibiotic residues in milk by BSTQ field kits was carried out quarterly in bulk milk samples from 10 villages. By extensive use of EVHP in these villages, in quarterly bulk milk sampling, presence of antibiotic residues of Betalactam gr., Sulphadimidine gr and for Quinolones gr. reduced substantially.

Globally, the use of antimicrobials in animals is nearly doubled than use in human (Aarestrup *et al,* 2012). Out of these, 30%–70% being released unaltered into the environment (Kummerer, 2001). In the present study, prior to EVHP awareness programme, around 70 % of the milk samples were tested positive for residues for at least one group of antibiotics. Within 12 months of study period with extensive awareness creation and practicing EVHP, the positivity of antibiotic residues was found to be coming down to around 10 % in the bulk milk samples of these DCSs.

The foremost reason of getting antibiotic residues in milk is injudicious usage of antibiotics in animals for treating infectious diseases (Zhang *et al.,* 2009). In analysing 224 articles across globe, Sachi *et al*., (2019) found that residues of β-lactam group have been most frequently detected followed by residues for other antibiotics viz., tetracyclines, fluoroquinolones, sulphonamides and aminoglycosides which is a serious public health concern. Antibiotic residues in milk i.e. for antibiotics like penicillin, tetracycline, oxytetracycline, beta lactam group, enrofloxacin, neomycin, streptomycin, sulphonamides, chloramphenicol, gentamicin has reported by several authors in India (Kalla *et al*., 2015, Kumarswamy *et al.,* 2018, Nirala *et al.,* 2017, Lunden, 2015, Lejaniya *et al.,* 2017, Gaurav *et al.,* 2014). Antibiotic residues in milk is suspected to have potential effects on public health. It may cause antibiotic resistance, allergic reactions, carcinogenicity, mutagenicity, teratogenicity and disturbances in the normal intestinal environment (Beyene, 2016).

Effect of using EVHP on SCC in bulk milk in villages at Sabarkantha Milk Union:

Screening for SCC in milk by field SCC Kits was carried out quarterly in bulk milk samples from 15 villages. The average SCC of the milk samples were found to be reduced from Initial 7.36 lakh/ml to 2.97 lakh/ml during the study period with extensive use of EVHP in the study areas.

Nair *et al.* (2017) also recorded external application of mixture of *Aloe vera* leaves, *Curcuma longa* rhizome and calcium hydroxide on udder also reduces SCC in mastitis cows. Mukherjee *et al*. (2005, 2010) also observed that use of EVHP can reduce SCC in sub-clinically infected cows.

Dermal safety study of EVHP Mastitis formulation:

EVHP for mastitis were applied on the skin of healthy young adult Wistar female rats, and the preparation was found to be non-toxic and no mortality was observed in these rats. No visible signs of toxicity such as edema, erythema, eschar formation, hyperkeratosis, hyperplasia, scaling, wound formation including no changes in respiration, circulation, autonomic and central nervous system, behavioural pattern was recorded during the entire observation period. Gross findings were agonal in nature and had no relation to treatment with the test substance.

Conclusion: In the present study, several bacterial agents were found to be involved in bovine clinical and subclinical mastitis as etiological agents with varying degrees of multidrug resistance. Increased awareness among dairy farmers and widespread usage of EVHP were found to be beneficial for both successful management of bovine mastitis and decreasing the use of antibiotics, which in turn reduced the degree of antibiotic residues in milk and improved quality in milk in terms of SCC. Additionally, empirical data suggest the immense value of EVHP in the effective treatment of many other diseases in dairy cows in addition to mastitis. Limited study indicates mastitis EVHP has no adverse effects on rats.

Further Scope of Study:

- Larger studies on the effect of use of EVHP in reduction of Antimicrobial Resistance (AMR).
- Management of other common bovine diseases by the use of EVHP in field condition.

References:

1. Aarestrup, F. (2012) Sustainable farming: get pigs off antibiotics. Nature. 486(7404):465–6
2. Aruna, M., Ambica, G., Lakshmi, K., Swathi, B., Padmaja, K. (2019) Efficacy of herbal preparations in the therapy of sub clinical mastitis in cows of periurban areas of Hyderabad. Pharma Innov J 8: 186–188
3. Beyene, T. (2016) Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. J Vet Sci Technol., 7(1):1–7
4. Bhatt, V.D., Shah, T.M., Nauriyal, D.S., Kunjadia, A.P., Joshi, C.G. (2014) Evaluation of a topical herbal drug for its in-vivo immunomodulatory effect on cytokines production and antibacterial activity in bovine subclinical mastitis. Ayu 35: 198–205

5. Chauhan, A., George, M., Chatterjee, P., Lindahl, J., Grace, D. (2018) The social biography of antibiotic use in smallholder dairy farms in India. Antimicrobial Resist Infect Control. 7(60). https://aricjournal.biomedcentral.com/track/pdf/10.1186/s13756-018-0354-9
6. Chelkeba, S.D., Misganaw, A.T., Efrem, A.G., Beza, E.E., Addisu, B.A. (2016) Adoption and impacts of dairy production technologies in Southwest Ethiopia: the cases of Jimma and Ilu-Ababora zones. *Journal of Biology, Agriculture and Healthcare.* 6 (7): 1-12.
7. Gaurav, A., Gill, J., Aulakh, R., Bedi, J. (2014) ELISA based monitoring and analysis of tetracycline residues in cattle milk in various districts of Punjab. Vet World., 7(1):26–9.
8. Hegde, R., Isloor, S., Nithin, Prabhu K., Shome, B.R., Rathnamma, D., Suryanarayana VV, *(2013)* Incidence of subclinical mastitis and prevalence of major mastitis pathogens in organized farms and unorganized sectors. Indian J Microbiol. 53: 315–320.
9. Hegde, Santhosha, Narendran, Pavithra, Gowda, Malali and Nair, M.N.B. (2021) Metagenomic Profiling of Bovine Milk from Mastitis Infected Udder of the Cows before and after Treatment with Ethno-Veterinary Practice (EVP). *EC Veterinary Science* 6.7 (2021): 71-80.
10. Kalla, A., Kutcharlapati, S., Gannavarapu, S., Layam, A. (2015) Isolation and identification of specific pathogens, presence of antibiotics, aflatoxins, pesticide residues and industrial contaminants in supply chain of milk in selected coastal districts of Andhra Pradesh. Adv Biosci Biotechnol. 6:330–44.
11. Kumarswamy., N., Latha, C., Menon, V., Sethukekshmi, C., Mercy, K. (2018) Detection of antibiotic residues in raw cow milk in Thrissur, India. Pharma Innov J., 7(8):452–4.
12. Kummerer, H.T.K. (2001) Pharmaceuticals in the environment – sources, fate, effects and risks. Aquat Toxicol., 71:391–2.
13. Lejaniya, A., Sathya, P., Sathian, C., Geetha, R., Radha, K. (2017) Screening of pooled milk samples for Beta lactam and tetracycline antibiotic residue. Int J Sci Environ Technol., 7(1):79–84.
14. Lunden, H. (2015) What's in the milk? Aflatoxin and antibiotic residues in cow's milk in Assam. https://stud.epsilon.slu.se/8196/7/lunden_h_160112.pdf.
15. Mukherjee, Reena, Dash, P.K., Ram, G.C. (2005) Immunotherapeutic potential of Ocimum sanctum (L) in bovine subclinical mastitis Research in Veterinary Science 79 (2005) 37–43.
16. Mukherjee, Reena, De, U. K., Ram, G. C. (2010) Evaluation of mammary gland immunity and therapeutic potential of Tinospora cordifolia against bovine subclinical mastitis. Trop Anim Health Prod, 42(4):645-51.
17. Mutua, F.; Sharma, G.; Grace, D.; Bandyopadhyay, S.; Shome, B.; Lindahl, J. (2020) A review of animal health and drug use practices in India, and their possible link to antimicrobial resistance. Antimicrob. Resist. Infect. Control, 9, 103.

18. NAAS (2013) Mastitis Management in Dairy Animals. Policy Paper No. 61, National Academy of Agricultural Sciences, New Delhi: 12 p.
19. Nair, M.N.B. and Punniamurthy, N. (2010) Contemporary relevance of Ethno veterinary practices. Preface. Proceedings of the international conference on "Ethno-Veterinary Practices: Mainstreaming traditional wisdom on livestock keeping and herbal medicine for sustainable rural livelihood. Jointly organised by TANUVAS and I-AIM at South Zone Cultural Centre, Thanjavur, India.
20. Nair. M. N. B., Punniamurthy, N., Mekala, P., Ramakrishnan, N. and Kumar, S.K. (2017). Ethnoveterinary Formulation for Treatment of Bovine Mastitis. Journal of Veterinary Sciences, pp 25-29.
21. Neelam, Jain, V.K., Singh, M., Joshi, V.G., Chhabra, R., Singh, K. (2022) Virulence and antimicrobial resistance gene profiles of *Staph. aureus* associated with clinical mastitis in cattle. PLoS ONE 17(5): e0264762.
22. Nirala, R., Anjana, K., Mandal, K., Jayachandran, C. (2017) Persistence of antibiotic residue in milk under region of Bihar, India. Int J Curr Microbiol App Sci., 6(3):2296–9.
23. Preethirani, P.L., Isloor, S., Sundareshan, S., Nuthanalakshmi, V., Deepthikiran, K., Sinha, A.Y. (2015) Isolation, Biochemical and Molecular Identification, and In-Vitro Antimicrobial Resistance Patterns of Bacteria Isolated from Bubaline Subclinical Mastitis in South India. PLoS ONE 10(11):e0142717.
24. Sachi, S., Ferdous, J., Sikder, M.H., Hussani, S.M.A.K. (2019) Antibiotic residues in milk: Past, present, and future. J Adv Vet Anim Res. 6(3):315–332.
25. Sadashiv, S. O. and Kaliwal, B. B. (2015) Screening and Antibiotic Resistance of *E. coli* Isolated from Bovine Mastitis in the region of North Karnataka, India. I*ndo American Journal of Pharm Research.2015:5(04).*
26. Satheshkumar, S., Punniamurthy, N., Ranganathan, V. (2021) 'Herbal Combo Therapy' for Oestrus Induction in Postpartum Anoestrus Cows. J Phytopharmacol 10(1):19-21.
27. Sharma, G.; Mutua, F.; Deka, R.P.; Shome, R.; Bandyopadhyay, S.; Shome, B.; Goyal Kumar, N.; Grace, D.; Dey, T.K.; Venugopal, N. (2020) A qualitative study on antibiotic use and animal health management in smallholder dairy farms of four regions of India. Infect. Ecol. Epidemiol., 10, 1792033.
28. Thorberg, B.M., Danielsson-Tham, M.L., Emanuelson, U., Persson Waller K. (2009) Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. J Dairy Sci., 92: 4962–4970.
29. Verma, R. K. (2014) An ethnobotanical study of plants used for the treatment of livestock diseases in Tikamgarh District of Bundelkhand, Central India. Asian Pac J Trop Biomed; 4 (Suppl 1): S460-S467
30. Yadav, R., Chhabra, R., Singh, M., Shrinet, G. and Talukdar, S.J. (2021). Antibiogram of *Kleb. pneumoniae* isolated from mastitic milk samples of cattle and buffalo. *Haryana Vet.* 60(2): 195-197.
31. Zhang, H., Ren, Y., Bao, X. (2009) Simultaneous determination of (fluoro) quinolones antibacterials residues in bovine milk using ultra performance liquid

chromatography–tandem mass spectrometry. J Pharm Biomed Anal 49(2):367–74.